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BIOCHEMICAL AND MORPHOMETRIC RELATIONSHIPS AMONG SOME MEMBERS OF THE CARDINALINAE

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ABSTRACT.—We used starch-gel electrophoresis to analyze relationships among 13 species in the subfamily Cardinalinae. These results were compared to a morphometric analysis of 15 skeletal characters and to a previous morphometric analysis of the subfamily by Hellack and Schnell (1977). Our results supported the phenetic classification of Hellack and Schnell and were more consistent with the classification of Hellmayr (1938) than with that of Paynter (1970). Divergence in morphometrics of skeletal characters in the Cardinalinae has been primarily in size. Electrophoretic data suggest that the Yellow-shouldered Grosbeak (*Caryothraustes humeralis*) is not a member of the Cardinalinae. In addition, if the broad genus *Saltator* is to be retained, then the genus *Pitylus* should be merged into it, and the Dickcissel (*Spiza americana*) is the outgroup to all other Cardinalinae examined (excluding *Caryothraustes humeralis*). *Received 22 Nov. 1991, accepted 15 April 1992.*

The subfamily Cardinalinae (Emberizidae/Fringillidae), consists of 37–42 species of cardinals, grosbeaks, and buntings (Paynter 1970, Morony et al. 1975, Sibley and Monroe 1990). Following a trend over the last three decades toward broader generic limits in avian systematics, seven genera (*Hedymeles, Richmondena, Pyrrhuloxia, Cyanocompsa, Cyanoloxia, Guiraca,* and *Porphyrospiza*) recognized by Hellmayr (1938) were merged into other genera by Paynter (1970). However, as has been typical of avian systematics throughout much of its history, reasons for taxonomic changes were not explicit.

Hellack and Schnell (1977) used plumage characters and skeletal measurements from Hellack (1976) to investigate phenetic relationships among the Cardinalinae. Although their phenetic classification resembles those of Hellmayr (1938), Paynter (1970), and Sibley and Monroe (1990), Hellack and Schnell's groupings were generally more similar to those of Hellmayr and of Sibley and Monroe than to those of Paynter. For example, morphometric results suggested that Paynter's expanded genus *Passerina* was a heterogenous and paraphyletic group (to the extent that overall similarity mirrors phylogeny). Hellack and Schnell's analysis also suggested that the genus *Saltator,* the limits of which have remained essentially unchanged during this century, might require revision.

With the advent of protein electrophoresis and other molecular-based

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techniques as tools for determining phylogenetic relationships, we have the opportunity to compare the results of genetic data to traditional, morphologically based classifications. We chose to focus on the Cardinalinae because Hellack and Schnell's classification provided an opportunity for comparison between a classification based on morphological characters with one based on allelic characters. We also sought to clarify the placement of the Yellow-shouldered Grosbeak (*Caryothraustes humeralis*), because several observers have noted that this species differs in some aspects of its natural history from other *Caryothraustes* species (Remsen and Ridgely 1980, Schulenberg et al. 1984, Remsen and Traylor 1989, Ridgely and Tudor 1989), providing further support for Hellmayr's (1938) concern that this species was not closely related to other *Caryothraustes*. Because skeletal material is now available for several taxa not available to Hellack and Schnell, we also include a morphometric analysis to examine the phenetic placement of these taxa.

MATERIALS AND METHODS

Electrophoresis.—Tissue samples from 27 individuals representing 16 taxa were collected between 1981–1985 by personnel of the Museum of Natural Science, Louisiana State Univ. *Atlapetes brunneinucha* (Fringillidae), *Catamblyrhynchus diadema* (Catamblyrhynchidae), and *Querula purpurata* (Cotingidae) were designated outgroups. The outgroup taxa vary in their phylogenetic relationships with the Cardinalinae, and these three outgroups were selected to maximize flexibility in the analyses and to allow comparisons between results generated by using different outgroups. Cardinaline taxa were selected to maximize potential comparisons with Hellack and Schnell's study by including at least one taxon from each of Hellack and Schnell's "best phenetic classification" clusters (Table 1). Generally, two individuals per taxon were examined. Thus, the number of species-level taxa was emphasized, rather than number of individuals per taxon, to estimate patterns of genetic variation among a variety of taxa.

Detailed procedures for collection, transport, and storage of tissues followed Johnson et al. (1984). Samples consisted of pooled portions of heart, liver, kidney, and pectoral muscle (<1.5 g total) minced and homogenized with an approximately equal volume of deionized water, and centrifuged at 12,000 rpm for 30 min. The supernatant was removed and stored at −70°C, and the pellet discarded. Standard horizontal starch-gel electrophoresis (Harris and Hopkinson 1976) was performed on the supernatant, using an 11.5% starch-gel and a variety of buffer systems (Tris-citrate II, pH 8.0; Lithium hydroxide, pH 8.0; phosphate citrate, pH 6.8; and Poulik, pH 8.7; see Selander et al. 1971).

Loci analyzed were: ADA (E.C. 3.5.4.4), AGPD (1.1.1.8), AK (2.7.4.3), CK (2.7.3.2), GDH (1.1.1.47), GOT1 (2.6.1.1), GOT2 (2.6.1.1), ICD1 (1.1.1.42), ICD2 (1.1.1.42), LDH (1.1.1.27), PEP-LGG (3.4.11), MDH1 (1.1.1.37), MDH2 (1.1.1.37), MPI (5.3.1.8), NP (2.4.2.1), PGI (5.3.1.9), PGM (2.7.5.1), 6PGD (1.1.1.44). Staining techniques and enzyme nomenclature follow Harris and Hopkinson (1976). Genotypic data for each individual at each locus were entered into Swofford and Selander's (1981) BIOSYS-1 computer program which was used to compute genetic distance coefficients of Nei (1978) and Rogers (1972), to derive distance Wagner trees, and to compile associated phenograms based on Rogers' (1972) genetic distance. Strict consensus trees were generated using PAUP (Swofford 1989), coding loci as characters and alleles as character states; the stability of the branching diagrams

TABLE 1

TAXA STUDIED, IDENTIFICATION NUMBER FOR PCA PLOTS, AND SAMPLE SIZES FOR THE SKELETAL AND ELECTROPHORETIC ANALYSES

Number	Species	Skeletal data		Electrophoresis
		N (♂)	N (♀)	N
1	Dickcissel (*Spiza americana*)	1	1	2
2	Yellow Grosbeak (*Pheucticus aureoventris*)	2	0	1
3	Yellow-bellied Grosbeak (*P. chrysogaster*)	0	1	0
4	Yellow Grosbeak (*P. chrysopeplus*)	2	0	0
5	Rose-breasted Grosbeak (*P. ludovicianus*)	6	3	2
6	Black-headed Grosbeak (*P. melanocephalus*)	2	2	0
7	Northern Cardinal (*Cardinalis cardinalis*)	10	10	0
8	Pyrrhuloxia (*C. sinuatus*)	2	0	2
9	Yellow-green Grosbeak (*Caryothraustes canadensis*)	2	2	0
10	Yellow-shouldered Grosbeak (*C. humeralis*)	1	0	1
11	Crimson-collared Grosbeak (*Rhodothraupis celaeno*)	0	2	0
12	Slate-colored Grosbeak (*Pitylus grossus*)	4	1	1
13	Streaked Saltator (*Saltator albicollis*)	2	2	2
14	Black-throated Saltator (*S. atricollis*)	2	0	0
15	Golden-billed Saltator (*S. aurantiirostris*)	4	4	2
16	Masked Saltator (*S. cinctus*)	0	1	0
17	Grayish Saltator (*S. coerulescens*)	6	3	2
18	Buff-throated Saltator (*S. maximus*)	7	6	2
19	Black-cowled Saltator (*S. nigriceps*)	0	1	0
20	Rufous-bellied Saltator (*S. rufiventris*)	2	0	0
21	Green-winged Saltator (*S. similis*)	1	0	0
22	Lazuli Bunting (*Passerina amoena*)	0	1	0
23	Ultramarine Grosbeak (*P. brissonii*)	1	1	0
24	Blue Grosbeak (*P. caerulea*)	2	2	2
25	Painted Bunting (*P. ciris*)	2	2	0
26	Indigo Bunting (*P. cyanea*)	2	2	2
27	Blue-black Grosbeak (*P. cyanoides*)	2	2	2
28	Indigo Grosbeak (*P. glaucocaerulea*)	1	1	0
29	Orange-breasted Bunting (*P. leclancherii*)	1	0	0
30	Blue Bunting (*P. parellina*)	0	1	0
31	Rose-bellied Bunting (*P. rositae*)	1	0	0
32	Varied Bunting (*P. versicolor*)	1	0	0
33	Rufous-sided Towhee (*Pipilo erythrophthalmus*)	1	1	0
34	Chipping Sparrow (*Spizella passerina*)	1	1	0
35	Purple-throated Fruitcrow (*Querula purpurata*)	0	0	1
36	Chestnut-capped Brush-finch (*Atlapetes brunneinucha*)	0	0	1
37	Plush-capped Finch (*Catamblyrhynchus diadema*)	0	0	1

was tested with the bootstrap procedure (Felsenstein 1985). Allelic frequency data generated from BIOSYS-1 were entered into the SAS (SAS Institute, Inc. 1985) program "PRINCOMP," which performed a principal components analysis (PCA) on arcsine square-root transformations of allelic frequencies. The transformations are intended to remove the dependence of the variance on the mean allelic frequency. Barrowclough and Johnson (1988) demonstrated the usefulness of executing a PCA on gene frequency data, showing that, unlike morphometric data, the genetic loci analyzed are the products of separate genomic sequences and thus are independent of each other. In light of this, PCA will not reduce the dimensionality of the data unless nongenetic factors, such as isolation, gene flow, selection, or "phylogenetic relatedness" influence many (or all) of the loci examined.

Morphometrics.—Table 1 lists all species analyzed, the code given to each, the number of skeletons, and the composition of samples with regard to sex. The 15 measurements selected were those found to be relatively repeatable (in the Fox Sparrow [*Passerella iliaca*]) by Zink (1983) and were chosen to represent a variety of regions, peripheral and core, of the bird skeleton. These measurements, made to the nearest 0.01 mm, are (1) SW = skull width, (2) SL = skull length, (3) CO = coracoid length, (4) SC = width of proximal end of scapula, (5) ST = sternum length, (6) PS = posterior synsacrum length, (7) SY = greatest width of synsacrum, (8) FP = width of proximal end of femur, (9) FD = width of distal end of femur, (10) FL = femur length, (11) TB = tibiotarsus length, (12) HT = head of trochanter (humerus), (13) HL = humerus length, (14) UL = ulna length, and (15) UP = width of proximal end of ulna. The majority of these measurements are described and illustrated by Robins and Schnell (1971).

These linear measurements were analyzed using univariate and multivariate techniques. To determine the feasibility of pooling sexes of the same species in the analyses, we quantified sexual dimorphism in the two species for which we had the largest samples, the Northern Cardinal (*Cardinalis cardinalis*) and (*Saltator aurantiirostris*). Analysis of variance (ANOVA) using SAS (SAS Institute, Inc. 1985) was used to analyze means of male, female, and pooled samples.

Taxonomic distances, calculated from standardized data, were used to construct UPGMA phenograms using the computer program "NTSYS" (Rohlf et al. 1974). Again, the analyses were performed on the three different data sets (male, female, and pooled). For each tree, a cophenetic correlation coefficient was calculated to indicate the goodness of fit of each tree to the original distance matrix (Rohlf et al. 1974).

Four taxa were represented by specimens lacking the rhampotheca: *Passerina rositae, P. glaucocaerulea, P. versicolor,* and *Rhodothraupis celaeno.* All analyses were performed with and without these taxa. In addition, the only character that would have been affected by the absence of the rhampotheca, skull length, was dropped in separate analyses. Results of these analyses failed to reveal any significant effect on the placement of the four taxa in question. However, omission of skull length did affect the placement of the other taxa. For this reason skull length was retained in the remaining analyses.

Two species of Emberizinae, a different subfamily within the Emberizidae, were included as "outgroups": Chipping Sparrow (*Spizella passerina*) and Rufous-sided Towhee (*Pipilo erythrophthalmus*). The Chipping Sparrow is similar in body size and bill shape to many cardinaline buntings, and the towhee is, likewise, superficially similar to many cardinaline grosbeaks and saltators.

Because taxa of the Cardinalinae range from 20 to 80 g in body mass, we attempted to correct for the effect of body size on the raw data. Body mass itself was not used because of its inherent variability in small samples and because it was not available for several taxa. Wiedenfeld (1978) found that for the Tyrannidae the length of the humerus was strongly correlated ($r = 0.98$) with cube root of body weight, and that this was the highest correlation

TABLE 2

DISTRIBUTION OF ELECTROMORPHS (DENOTED BY LOWER CASE LETTERS) IN 13 CARDINALINAE AND ONE OUTGROUP TAXON (*QUERULA PURPURATA*)

Species	Locus[a]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Spiza americana	ee	e	a	a	a	ac	a	d	b	a	e	a	a	a	df	a	b	d
Pheuticus aureoventris	e	a	b	a	a	e	a	a	b	a	e	a	a	a	d	a	b	e
P. ludovicianus	c	a	b	a	a	e	a	b	b	a	e	a	a	a	d	a	b	be
Cardinalis sinuatus	e	a	b	a	a	e	a	b	b	a	e	a	a	a	e	a	b	c
Caryothraustes humeralis	d	e	a	a	a	b	a	b	a	a	d	a	a	a	a	a	e	e
Pitylus grossus	e	b	b	a	a	e	a	d	b	a	e	a	a	a	bd	a	ae	b
Saltator albicollis	e	b	b	a	a	c	a	ed	b	a	ae	a	a	a	e	a	e	b
S. aurantiirostris	e	b	b	a	a	e	a	b	b	a	e	a	a	a	e	a	e	b
S. coerulescens	e	b	b	a	a	e	a	d	b	a	e	a	a	a	e	a	c	ab
S. maximus	e	b	b	a	a	e	a	e	b	a	ce	a	a	a	ce	a	be	b
Passerina caerulea	e	b	b	a	a	c	a	b	b	a	e	a	a	a	d	a	b	e
P. cyanea	b	b	b	a	a	a	a	b	b	a	c	a	a	a	d	a	b	c
P. cyanoides	e	b	b	a	a	e	a	b	a	a	ae	a	a	ab	d	a	b	bc
Querula purpurata	a	d	c	a	a	d	b	e	c	a	b	a	a	e	d	a	b	e

[a] Loci in order are: ADA, AGDP, AK, CK, GDH, GOT1, GOT2, ICD1, ICD2, LDH, PEP-LGG, MDH1, MDH2, MPI, NP, PGI, PGM, 6PGD.

with weight among 58 skeletal characters. By using the cube root, weight was transformed to a linear variable comparable to humerus length (Wiedenfeld 1978). To determine if the relationship between humerus length and cube root of body mass holds for the Cardinalinae, we calculated the regression coefficient of humerus length on the cube root of weight. This regression indicated a strong relationship ($r = 0.91$). Using humerus length to estimate body size, linear regressions were performed with humerus length as the independent variable and all other characters as dependent variables. Following Reist's (1986) method for size correction, residuals were used as raw data in the same analyses described above.

RESULTS

Electrophoresis.—Eighteen loci were identified for each of the individuals sampled, of which five were monomorphic, six showed few (2–3) alleles, and seven were highly variable. The distribution of alleles for 18 loci among the Cardinalinae taxa and one outgroup taxon is presented in Table 2. Nei's (1978) genetic distance values within the Cardinalinae range from 0.019 to 0.732 (Table 3). Rogers' (1972) genetic distance values within the Cardinalinae range from 0.056 to 0.529 (Table 3). The average

TABLE 3
MATRIX OF GENETIC DISTANCE COEFFICIENTS[a]

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *Spiza americana*		0.328	0.484	0.383	0.347	0.286	0.292	0.383	0.269	0.347	0.295	0.376	0.356	0.558
2 *Passerina cyanea*	0.362		0.444	0.222	0.278	0.181	0.111	0.278	0.300	0.326	0.328	0.333	0.278	0.556
3 *Caryothraustes humeralis*	0.650	0.588		0.425	0.500	0.458	0.444	0.444	0.506	0.529	0.495	0.465	0.444	0.667
4 *Passerina cyanoides*	0.400	0.203	0.515		0.258	0.153	0.111	0.258	0.328	0.335	0.356	0.333	0.306	0.537
5 *Pheucticus aureoventris*	0.383	0.325	0.693	0.258		0.125	0.167	0.111	0.356	0.298	0.383	0.374	0.389	0.556
6 *P. ludovicianus*	0.287	0.185	0.599	0.128	0.119		0.069	0.125	0.286	0.312	0.315	0.319	0.264	0.550
7 *Passerina caerulea*	0.301	0.118	0.588	0.069	0.182	0.058		0.167	0.245	0.270	0.272	0.278	0.222	0.556
8 *Cardinalis sinuatus*	0.449	0.325	0.588	0.258	0.118	0.119	0.182		0.376	0.278	0.328	0.298	0.278	0.611
9 *Pitylus grossus*	0.258	0.331	0.688	0.323	0.415	0.297	0.254	0.459		0.173	0.076	0.138	0.118	0.633
10 *Saltator maximus*	0.356	0.357	0.732	0.335	0.316	0.322	0.277	0.277	0.138		0.181	0.134	0.167	0.557
11 *S. coerulescens*	0.307	0.391	0.679	0.391	0.478	0.362	0.311	0.391	0.039	0.136		0.090	0.069	0.661
12 *S. albicollis*	0.410	0.367	0.601	0.339	0.454	0.332	0.287	0.326	0.094	0.071	0.050		0.056	0.631
13 *S. aurantiirostris*	0.405	0.325	0.588	0.316	0.492	0.292	0.521	0.325	0.100	0.134	0.058	0.019		0.667
14 *Querula purpurata*	0.799	0.811	1.099	0.762	0.811	0.797	0.811	0.944	0.985	0.794	1.085	0.980	1.099	

[a] Rogers' (1972) genetic distance above diagonal, Nei's (1978) unbiased genetic distance below diagonal.

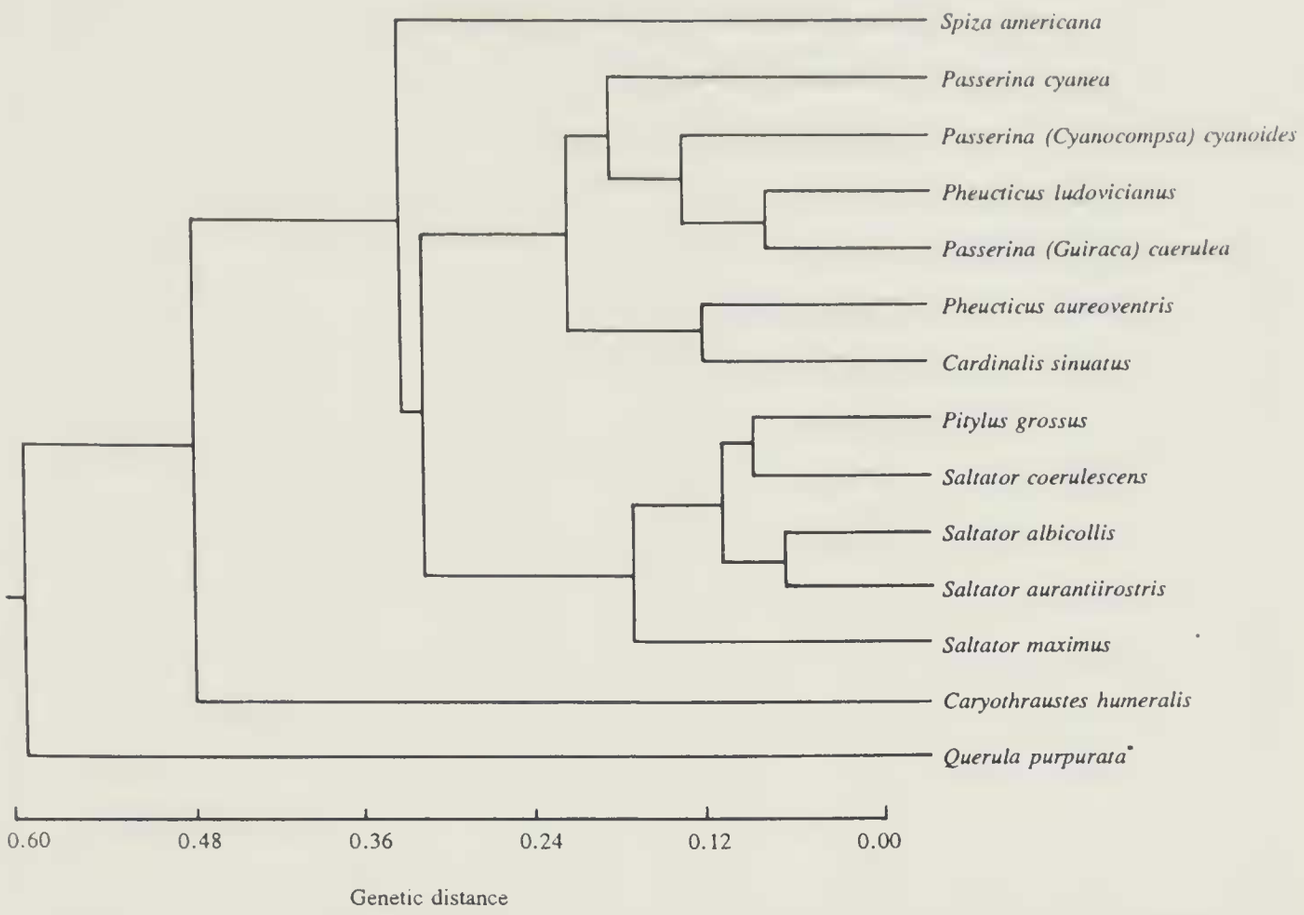

FIG. 1. UPGMA phenogram derived from Rogers' (1972) genetic distance matrix. The cophenetic correlation coefficient is 0.964. Asterisk (*) denotes outgroup.

intrageneric genetic distance values ($\bar{D}$) are: *Saltator* = 0.066 (N = 4); *Passerina* = 0.013 (N = 3); and *Pheucticus* = 0.119 (N = 2). The average genetic distance between members of the subfamily is 0.324; however, computing $\bar{D}$ for the subfamily excluding *Caryothraustes humeralis* reduces the value to 0.269.

A UPGMA phenogram (Fig. 1) reveals relationships that are fundamentally similar to Hellack and Schnell's (1977) "best phenetic classification." *Querula purpurata* was chosen as the outgroup in the distance analysis because it displayed the most unique alleles among the potential outgroups and it clarified the branching diagrams. Most noteworthy is the removal of *Caryothraustes humeralis* from the Cardinalinae lineage. The other major difference is the placement of *Cardinalis sinuatus*, which although phenetically linked to the *Saltator* complex, clusters genetically with the *Passerina-Pheucticus* complex.

A 50% majority rule consensus tree, 49 steps long, was produced from 172 most parsimonious trees (Fig. 2). Consensus trees were generated using *Atlapetes brunneinucha, Catamblyrhynchus diadema*, and *Querula purpurata* as outgroups. These trees show similarities to each other as well as with the UPGMA phenogram (Fig. 1). The major differences between the consensus tree with *Q. purpurata* as the outgroup and Fig. 1

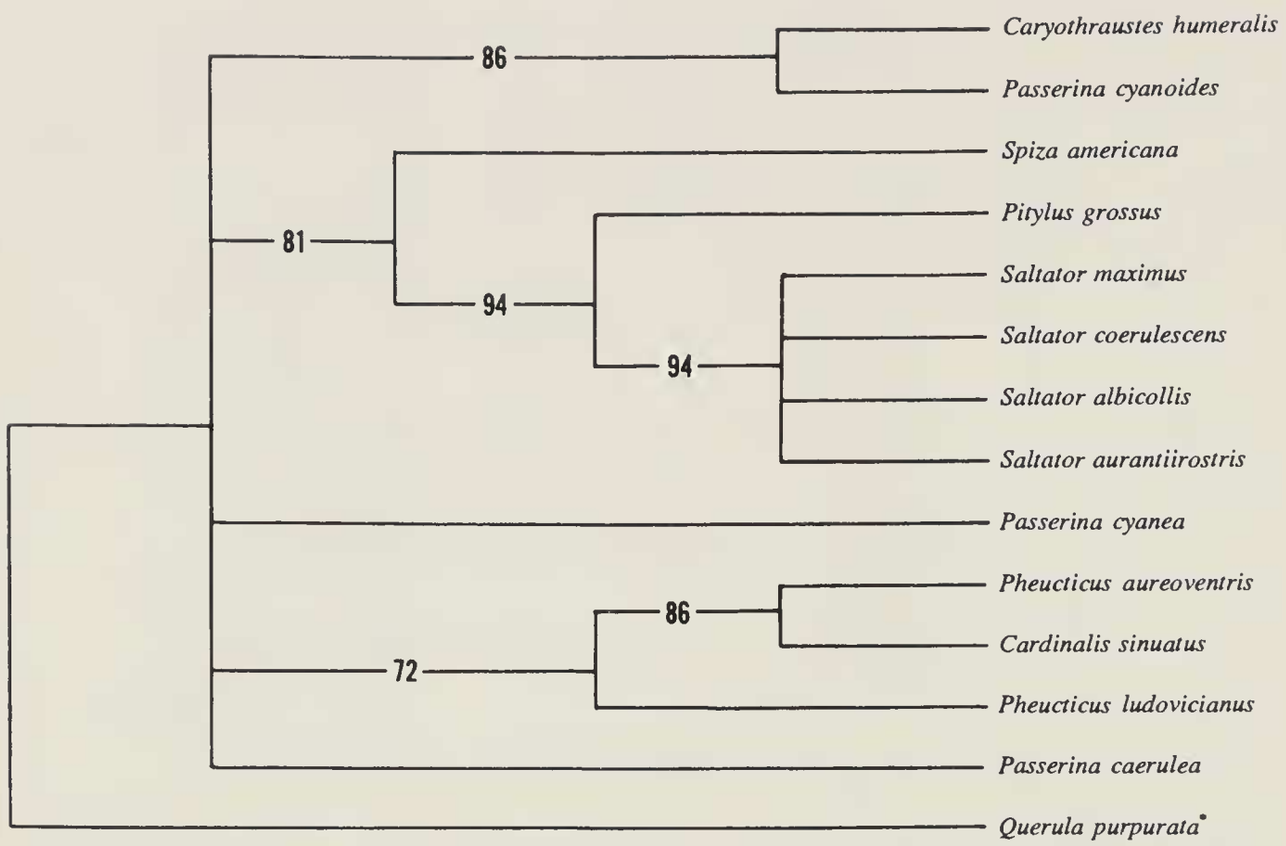

FIG. 2. Strict consensus tree of 14 taxa. The length of the tree is 49 steps, and the consistency index is 0.677, excluding uninformative characters. The numbers indicated on the branches correspond to the percentage of 172 most parsimonious trees which support the placement of that branch. Asterisk (*) denotes outgroup.

were the placement of *Passerina* (*Cyanocompsa*) *cyanoides,* which clusters with *Caryothraustes humeralis* due to the presence of a synapomorphy at the ICD-2 locus. *Caryothraustes humeralis,* however, shows eight autapomorphies, which accounts for its removal from the subfamily in the distance analysis. In addition, the placement of *Spiza americana* within the *Pitylus-Saltator* complex contrasts with its outlying position in Fig. 1. A strict consensus tree, 41 steps long, with *Atlapetes brunneinucha* as the outgroup is nearly identical to Fig. 2. The consensus tree with *C. diadema* as the outgroup is identical to Fig. 2, with the exception of *Spiza americana,* which is the outlier to all Cardinalines in both this tree as well as in Fig. 1 (with the exception of *Caryothraustes humeralis*). A strict consensus tree 50 steps long was generated using both *A. brunneinucha* and *Q. purpurata* as outgroups. This tree is identical (with the addition of *Q. purpurata*) to the consensus tree with *Atlapetes* as the only outgroup. Using different outgroups and combinations of outgroups had little effect on the results of the cladistic analyses. Cladistic analyses of allozyme data are compromised, however, because they do not consider gene frequency information (Swofford and Berlocher 1987). Bootstrapping (Felsenstein 1985) the consensus trees collapsed all nodes of the branching diagram,

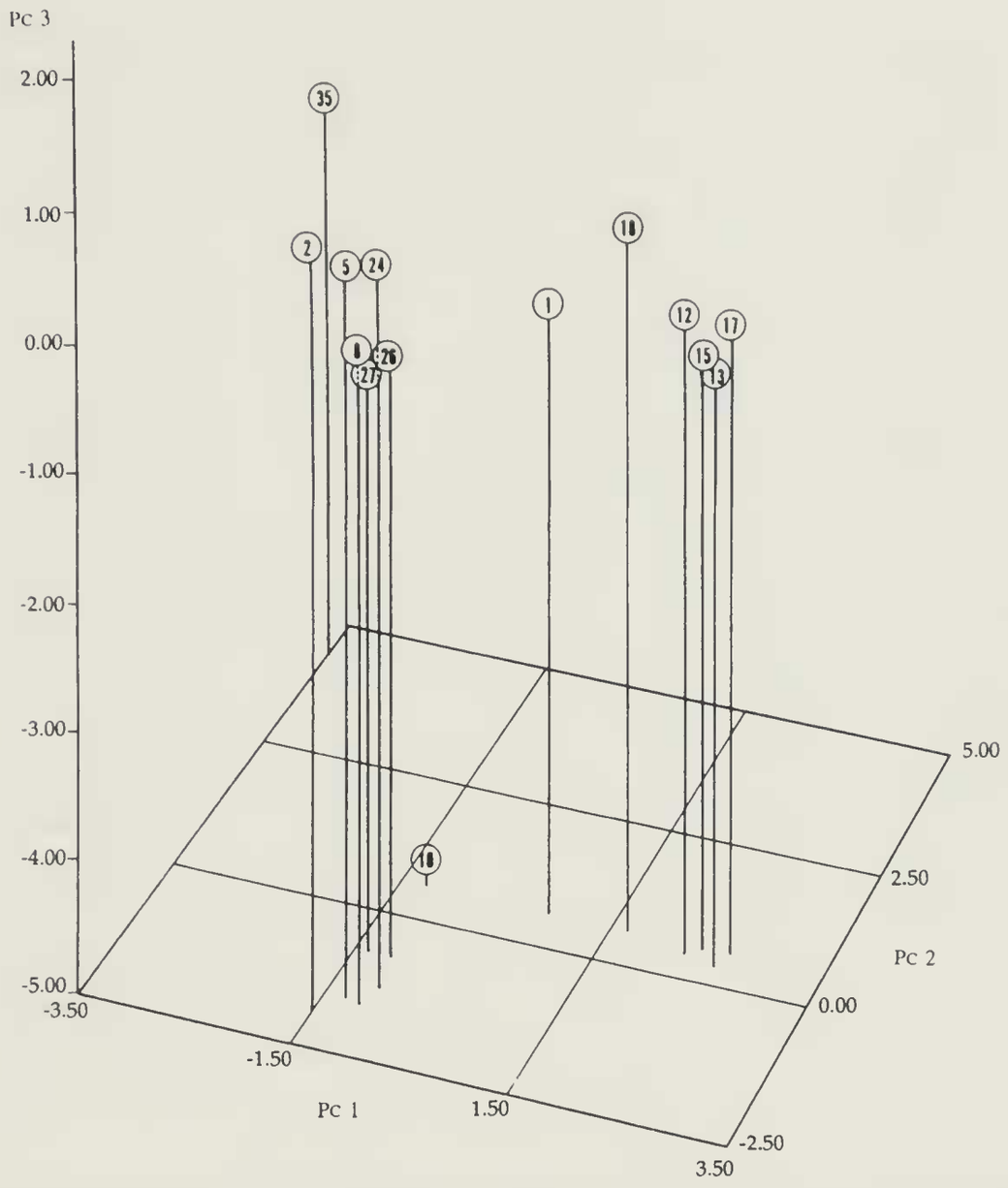

FIG. 3. Distribution of 14 taxa across Principal Components 1, 2, and 3 based on electrophoretic data. Species numbers are: (1) *Spiza americana*; (2) *Pheucticus aureoventris*; (5) *P. ludovicianus*; (8) *Cardinalis sinuatus*; (10) *Caryothraustes humeralis*; (12) *Pitylus grossus*; (13) *Saltator albicollis*; (15) *S. aurantiirostris*; (17) *S. coerulescens*; (18) *S. maximus*; (24) *Passerina caerulea*; (26) *P. cyanea*; (27) *P. cyanoides*; (35) *Querula purpurata*.

except the branches associated with the outgroup(s), resulting in one large Cardinalinae cluster.

Principal component 1, which explains 28% of the total variation, separates the *Saltator-Pitylus* complex from the *Pheucticus-Passerina* cluster and demonstrates the distinctiveness of the outgroup *Querula purpurata* (Fig. 3). Principal component 2 explains 21% of the total variance and serves chiefly to isolate the outgroup from the Cardinalinae, although the *Passerina-Pheucticus* grouping appears well-removed from the remaining taxa. Principal component 3 (16% of the total variance) shows marked

disjunction between *Caryothraustes* and other Cardinalinae, and further contributes to isolating the outgroup. Thus, in the plot of the first three components, which accounts for nearly 65% of the total variance, *Caryothraustes humeralis* and *Querula purpurata* are clearly distinct from the other Cardinalinae, as well as from each other. Loci showing substantial contributions of the original variables to the PC axes are: PGM, ADA, 6GPD, and NP (PC 1); ICD1, GOT1, GOT2, MPI, AK, and AGPD (PC 2); and ICD2, PGM, NP, PEP-LGG and AK (PC 3).

Morphometrics.—ANOVA revealed pronounced sexual dimorphism in skeletal characters. In *Cardinalis cardinalis,* 10 of 15 characters differed significantly ($P < 0.05$), with the males being larger. In *Saltator aurantiirostris,* 11 characters differed significantly, with the females having larger values. Differences of this magnitude, producing bimodality of the sample means, indicate that the sexes should not be pooled (Wallace 1984). Because of limited sample sizes, Hellack and Schnell (1977) were forced to pool sexes in several species.

The amount of variation described by PC 1 in the combined male and female PCA uncorrected for size ranged from 88.6% in females to 97.4% in males. PC 2 described from 2.0% to 5.4% and PC 3, from 0.8% to 2.7%. Character loadings were equal and positive on PC 1; this suggests that PC 1 is strongly related to body size (Wiley 1981, Freeman and Jackson 1990). Examination of PCA plots (Figs. 4 and 5) further indicates that the effect of size on this analysis is great. Smaller species in the expanded genus *Passerina* are found on the low end of PC 1, whereas the largest saltators and grosbeaks are on the higher end.

UPGMA phenograms without size correction (Figs. 6 and 7) also show the effects of body size. The smaller *Spizella passerina* tends to cluster with the smaller cardinalines and *Pipilo erythrophthalmus* clusters with some saltators and other large species.

In analyses with the data standardized for body size (Fig. 8), PC 1 describes from 28% to 33% of the total variation, PC 2 ranges from 20% to 33%, and PC 3 describes 13% in all three plots. The total variation described by the plots is 61% for the combined samples, 63% for the male samples, and 69% for the female samples.

After correction for body size, the two outgroups are well separated from the main group, although *Saltator nigriceps* is found near *Pipilo erythrophthalmus* in both the plots of females and the combined samples. *Spiza americana* is also found outside the subfamily. *Pheucticus melanocephalus* and *P. ludovicianus* are close together as would be expected of two allospecies that hybridize (West 1962). However, the allospecies *P. chrysogaster* and *P. chrysopeplus* did not cluster together, presumably because these taxa are only represented by female and male samples respectively. The *Cardinalis* samples, however, do not cluster together in

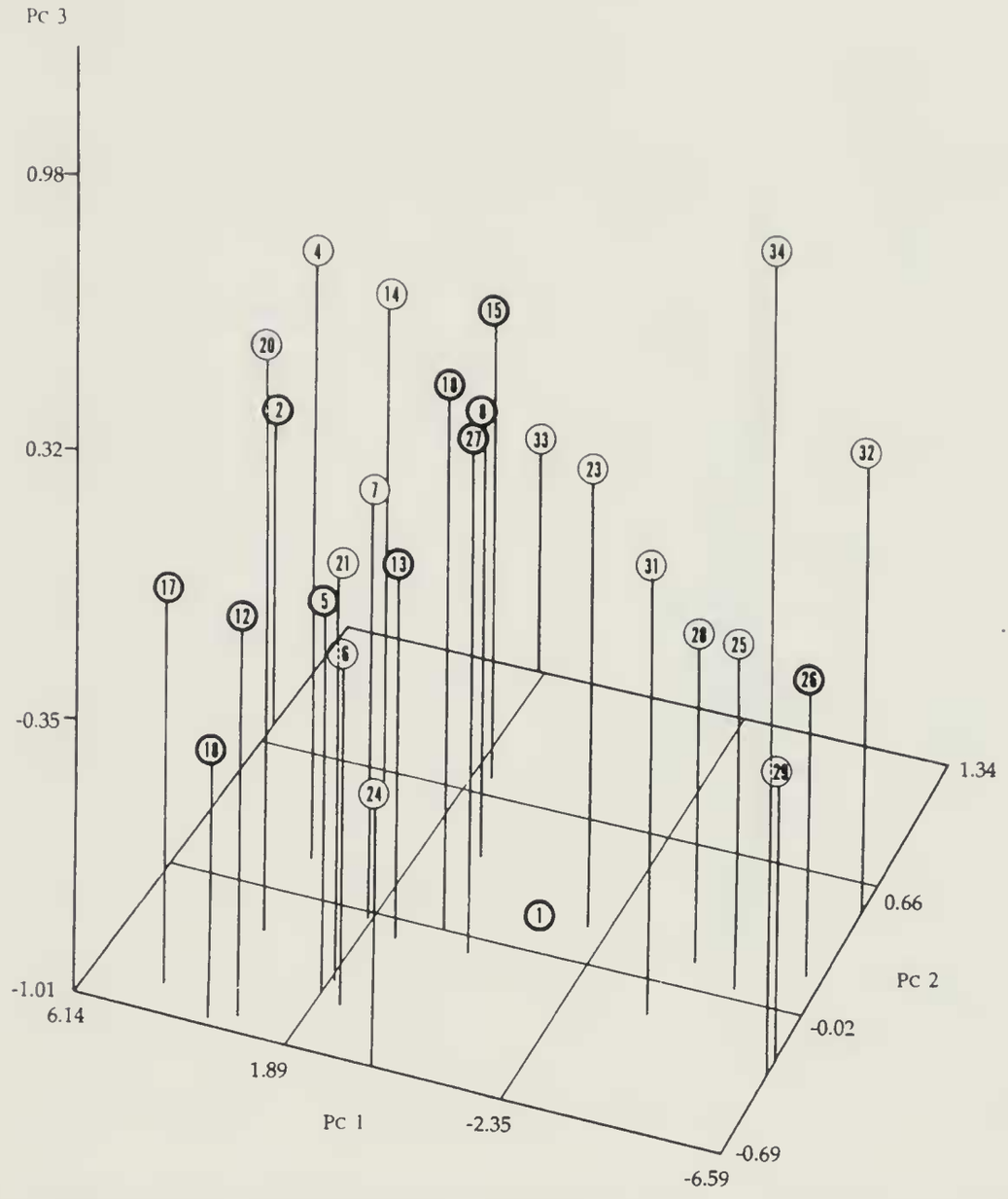

FIG. 4. Distribution of 28 taxa across Principal Components 1, 2, and 3 based on uncorrected morphometric data of male specimens only. Species numbers are: (1) *Spiza americana*; (2) *Pheucticus aureoventris*; (4) *P. chrysopeplus*; (5) *P. ludovicianus*; (6) *P. melanocephalus*; (7) *Cardinalis cardinalis*; (8) *C. sinuatus*; (9) *Caryothraustes canadensis*; (10) *C. humeralis*; (12) *Pitylus grossus*; (13) *Saltator albicollis*; (14) *S. atricollis*; (15) *S. aurantiirostris*; (17) *S. coerulescens*; (18) *S. maximus*; (20) *S. rufiventris*; (21) *S. similis*; (23) *Passerina brissonii*; (24) *P. caerulea*; (25) *P. ciris*; (26) *P. cyanea*; (27) *P. cyanoides*; (28) *P. glaucocaerulea*; (29) *P. leclancherii*; (31) *P. rositae*; (32) *P. versicolor*; (33) *Pipilo erythrophthalmus*; and (34) *Spizella passerina*. Bold circles indicate taxa used in the electrophoretic analysis.

the plot of male-only samples. *Caryothraustes humeralis* is found within the main group but not near its supposed congener *C. canadensis*.

The combined male and female UPGMA phenogram (Fig. 9) corrected for size gives results similar to those of the size corrected PCA. Both outgroups cluster outside most Cardinalinae, although *S. nigriceps* again is also found outside the subfamily. *Caryothraustes humeralis* is in the main group, but again apart from *C. canadensis*. The cardinals remain a distinct cluster. *Pheucticus melanocephalus* and *P. ludovicianus* again clus-

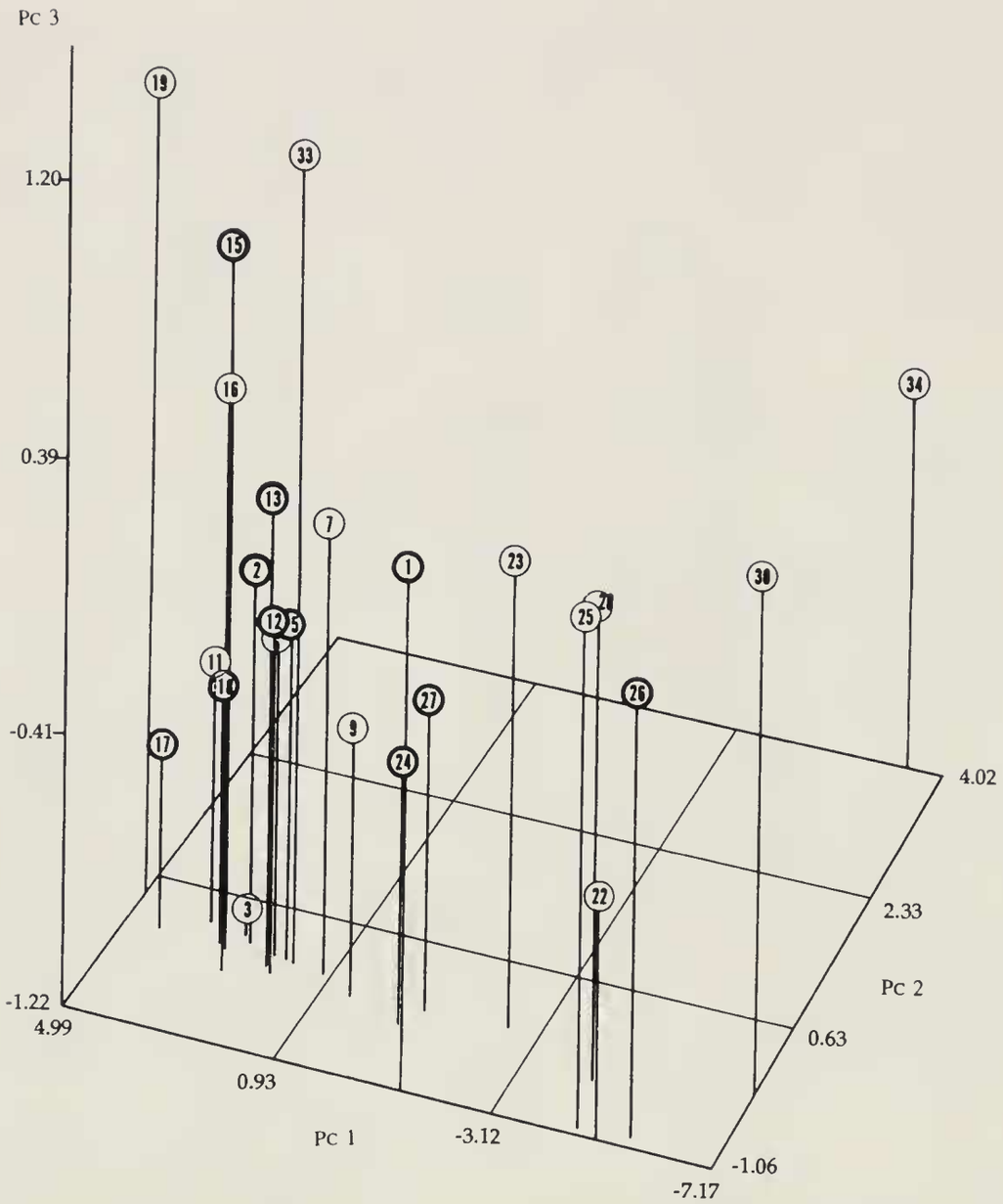

FIG. 5. Distribution of 24 taxa across Principal Components 1, 2, and 3 based on uncorrected morphometric data of female specimens only. Species numbers are: (1) *Spiza americana*; (3) *Pheucticus chrysogaster*; (5) *P. ludovicianus*; (6) *P. melanocephalus*; (7) *Cardinalis cardinalis*; (9) *Caryothraustes canadensis*; (11) *Rhodothraupis celaeno*; (12) *Pitylus grossus*; (13) Saltator albicollis; (15) *S. aurantiirostris*; (16) *S. cinctus*; (17) *S. coerulescens*; (18) *S. maximum*; (19) *S. nigriceps*; (22) *Passerina amoena*; (23) *P. brissoni*; (24) *P. caerulea*; (25) *P. ciris*; (26) *P. cyanea*; (27) *P. cyanoides*; (28) *P. glaucocaerulea*; (30) *P. parellina*; (33) *Pipilo erythrophthalmus*; and (34) *Spizella passerina*. Bold circles indicate taxa used in the electrophoretic analysis.

ter closely. Species from two large genera, Passerina and Saltator, show little tendency to cluster together.

Results from the analyses without size-correction resemble both the Hellack-Schnell and the biochemical classifications. However, our results indicate that both *Passerina* (*Guiraca*) *caerulea* and *P.* (*Cyanocompsa*) *cyanoides* lie outside the main group of cardinalines along with *Caryothraustes,* whereas Hellack and Schnell placed *P. caerulea* outside *Pas-*

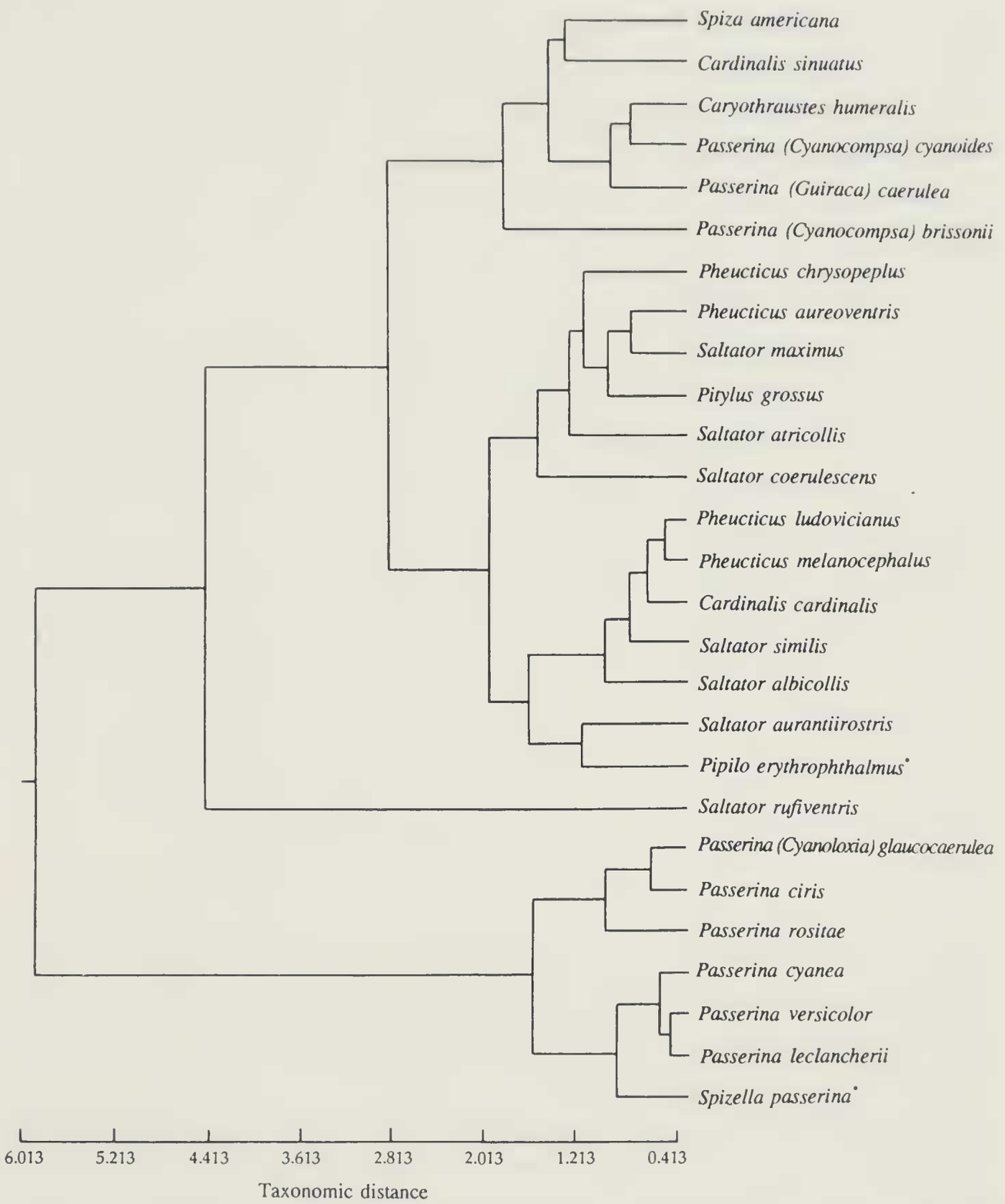

FIG. 6. UPGMA phenogram derived from uncorrected morphometric data based on male specimens only. The cophenetic correlation coefficient is 0.832. Asterisk (*) denotes outgroups.

serina. In addition, *P. brissonii* clusters with *P. caerulea* and *P. cyanoides* in the separate phenograms for the sexes. Hellack and Schnell's results also suggest that *Cardinalis* and *Pheucticus* contain species that morphologically are more similar to each other than to species in other genera, whereas our results do not indicate this. The fragmented nature of the

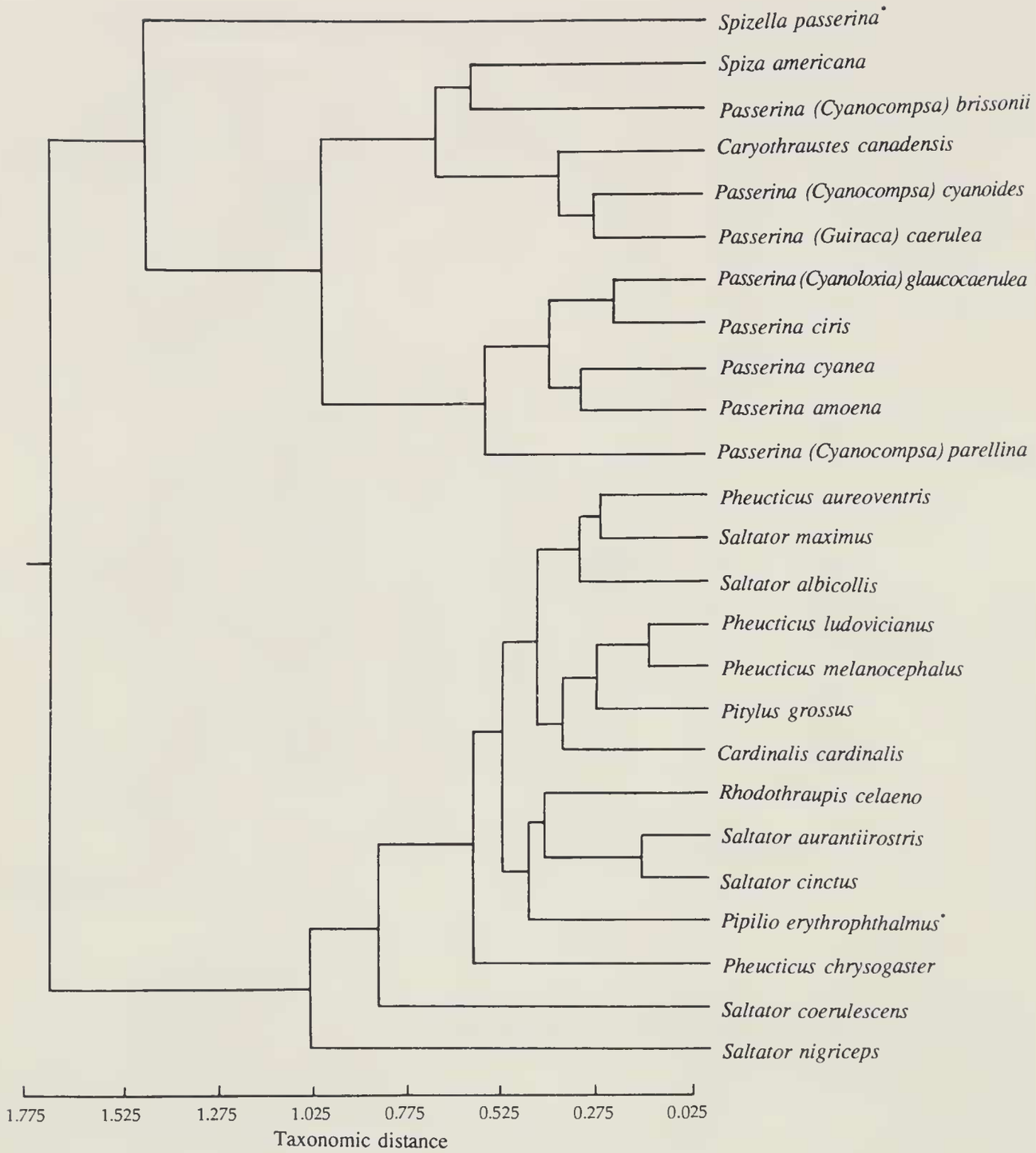

FIG. 7. UPGMA phenogram derived from uncorrected morphometric data based on female specimens only. The cophenetic correlation coefficient is 0.759. Asterisk (*) denotes outgroups.

genus *Saltator* results from the inclusion of many taxa not included in Hellack and Schnell's analysis of skeletal characters. The clustering of *Pitylus grossus* and *Rhodothraupis celaeno* within the saltators agrees with Hellack and Schnell's (1977) results. The results of the size-corrected phenograms agree with our biochemical results on the distinctiveness of *Spiza americana.* However, the separation of two pairs of hybridizing allospecies (*Passerina cyanea* and *P. amoena; Pheucticus ludovicianus* and

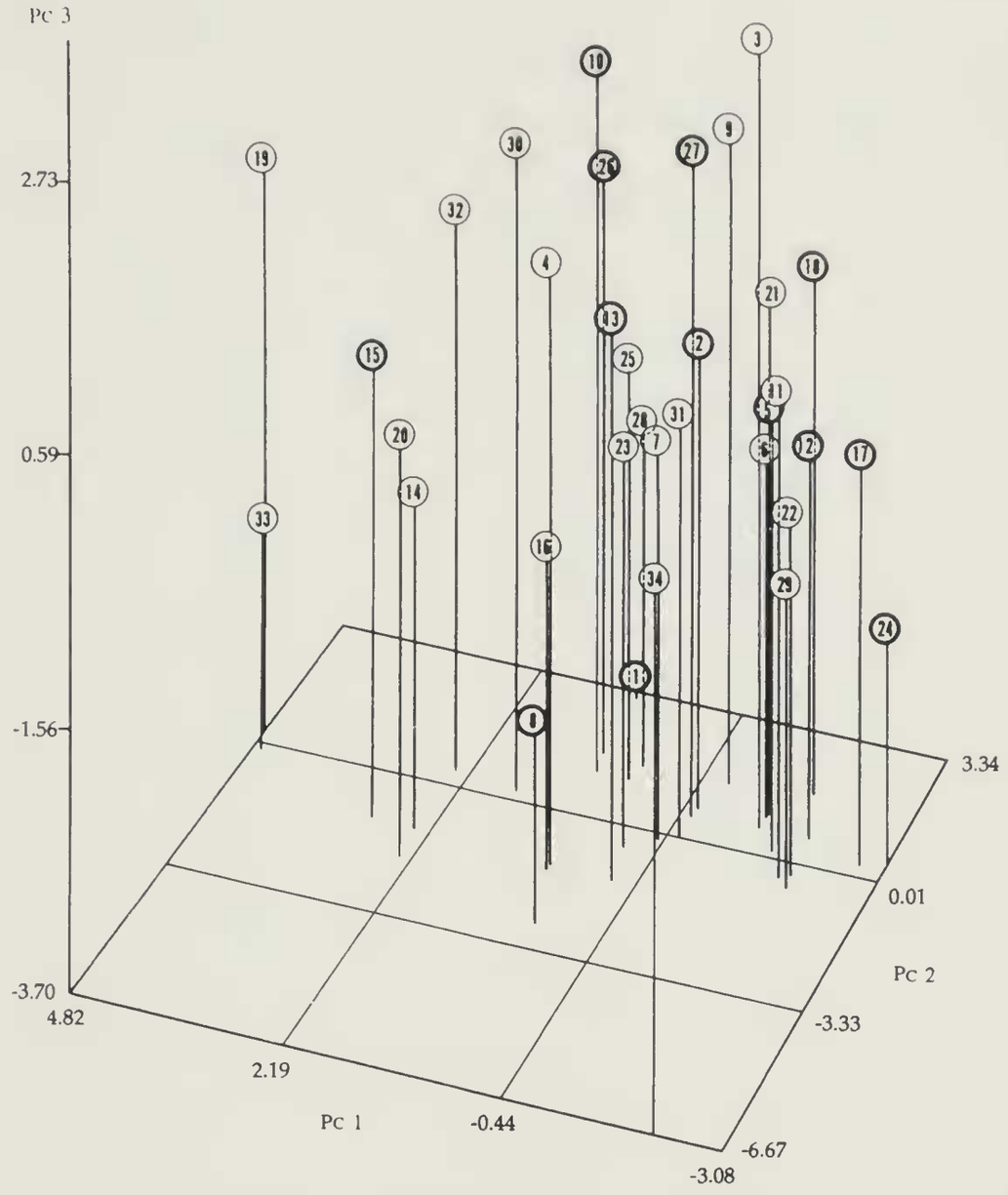

FIG. 8. Distribution of 34 taxa across Principal Components 1, 2, and 3 based on size-corrected morphological data of both males and females. Species numbers are: (1) *Spiza americana*; (2) *Pheucticus aureoventris*; (3) *Pheucticus chrysogaster*; (4) *P. chrysopeplus*; (5) *P. ludovicianus*; (6) *P. melanocephalus*; (7) *Cardinalis cardinalis*; (8) *C. sinuatus*; (9) *Caryothraustes canadensis*; (10) *C. humeralis*; (11) *Rhodothraupis celaeno*; (12) Pitylus grossus; (13) *Saltator albicollis*; (14) *S. atricollis*; (15) *S. aurantiirostris*; (16) *S. cinctus*; (17) *S. coerulescens*; (18) *S. maximus*; (19) *S. nigriceps*; (20) *S. rufiventris*; (21) *S. similis*; (22) *Passerina amoena*; (23) *P. brissoni*; (24) *P. caerulea*; (25) *P. ciris*; (26) *P. cyanea*; (27) *P. cyanoides*; (28) *P. glaucocaerulea*; (29) *P. leclancherii*; (30) *P. parellina*; (31) *P. rositae*; (32) *P. versicolor*; (33) *Pipilo erythrophthalmus*; and (34) *Spizella passerina*. Bold circles indicate taxa used in the electrophoretic analysis.

P. melanocephalus) indicates problems with phylogenetic interpretations of these results.

DISCUSSION

The results of our morphometric analysis present a problem to systematists who would use morphometrics to infer phylogeny. If correction for

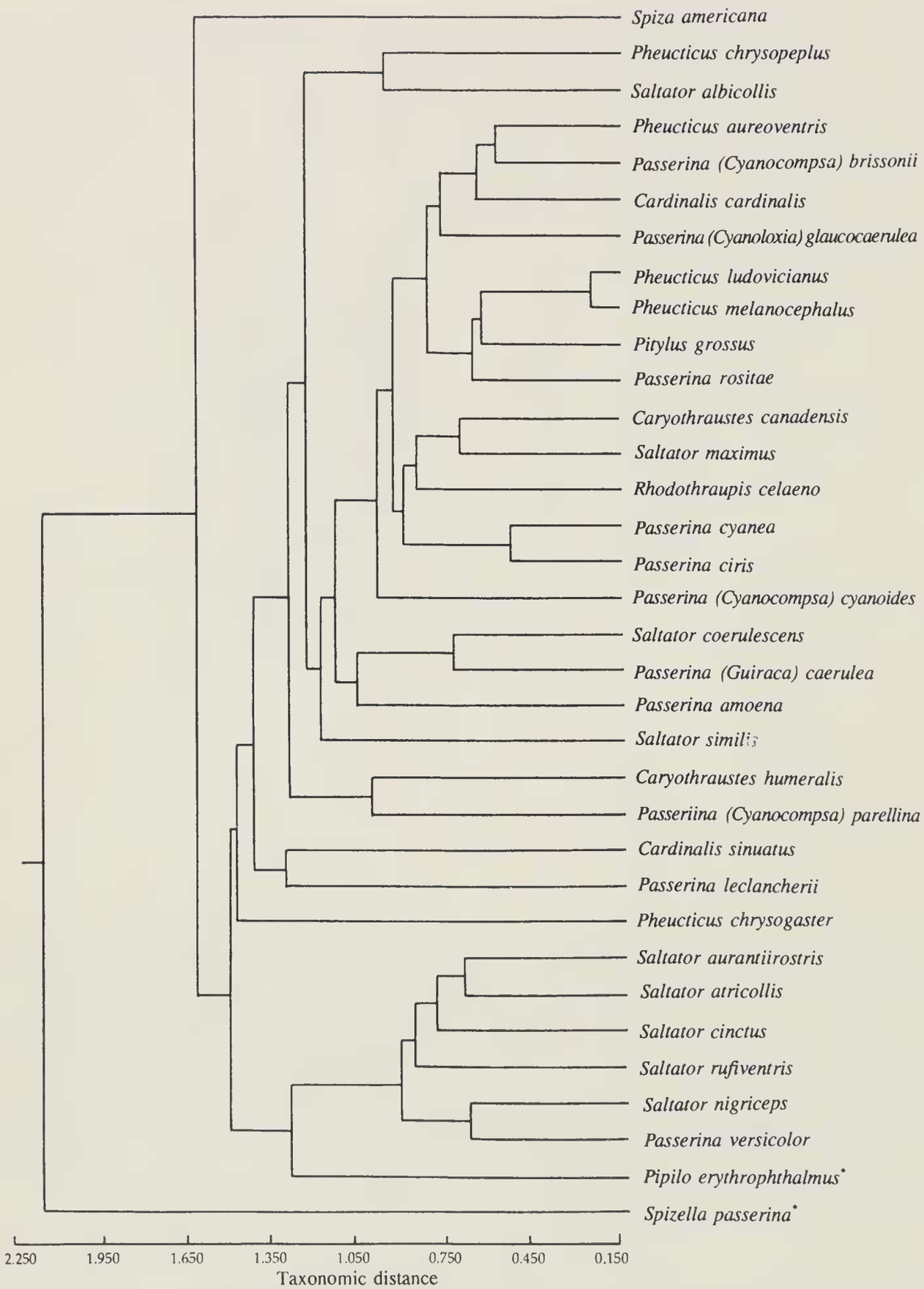

FIG. 9. UPGMA phenogram derived from size-corrected morphological data of both sexes. The cophenetic correlation coefficient is 0.789. Asterisk (*) denotes outgroups.

size is not made, then it is impossible to distinguish outgroups. However, if correction for size is made, then the resulting phenogram makes little sense. For example, the phenogram places two hybridizing allospecies, *Passerina amoena* and *P. cyanea* (Sibley and Short 1959), in different clusters. Phenetic analyses of morphometric data may not be able to separate outgroups when variability in size is large among the taxa under investigation and when shape differences are small, as in the Cardinalinae, which seem to have undergone little divergence in shape. On the other hand, our results suggest that size itself has a phylogenetic basis within the Cardinalinae. Although we cannot rule out the possibility that measurement of additional characters would have provided more resolution, most morphometric analyses of birds have shown that most skeletal characters, particularly in the trunk skeleton, are highly autocorrelated and contribute little additional information.

Relationships of Passerina, Guiraca, and Cyanocompsa.—Hellmayr (1938) and Hellack and Schnell (1977) favored retention of all three genera, whereas Paynter (1970) merged *Guiraca* and *Cyanocompsa* in *Passerina* without comment. Because biochemical data suggest that *Passerina* (*Guiraca*) *caerulea* might be more closely related to *Pheucticus ludovicianus* than to either *Passerina cyanea* or *Passerina* (*Cyanocompsa*) *cyanoides,* our results support the classifications of Hellmayr (1938), Hellack and Schnell (1977), the AOU (1983), Ridgely and Tudor (1989), and Sibley and Monroe (1990), in retaining the genera *Guiraca* and *Cyanocompsa.* Unfortunately, tissue samples for the Blue Bunting (*Passerina* [*Cyanocompsa*] *parellina*), the Indigo Grosbeak (*Passerina* [*Cyanoloxia*] *glaucocaerulea*), and the Blue Finch (*Passerina* [*Porphyrospiza*] *caerulescens*) were not available. Ridgely and Tudor (1989) removed the latter species from the Cardinalinae, a change supported by Bates et al. (in press).

Monophyly of Pheucticus.—Hellack and Schnell (1977) found that the genus *Pheucticus* as viewed by Paynter (1970) consisted of two rather different groups, one of which was more closely related to *Guiraca* (see above). Hellmayr (1938) had previously separated these two groups into two genera, retaining *Hedymeles* for *ludovicianus* and *melanocephalus.* Our results, both biochemical and morphometric, support the classifications of Hellmayr and Hellack and Schnell. Paynter's broader *Pheucticus* may be paraphyletic, with *Guiraca* seemingly more closely related to "*Pheucticus*" *ludovicianus* and *Cardinalis cardinalis* more closely related to *P. aureoventris.* However, the difference in allozymes is so small among these taxa that we hesitate to recommend resurrection of *Hedymeles* for *ludovicianus* and *melanocephalus* without corroborating data from other kinds of analyses. The strong plumage and vocal similarities between

ludovicianus and *melanocephalus* and the *aureoventris* group (Ridgely and Tudor 1989; Remsen, pers. obs.) seem unlikely to be merely shared primitive characters. Likewise, the strong plumage and vocal similarities of *Guiraca caerulea* to the *Passerina* buntings seem unlikely to be merely shared primitive characters. These similarities, obviously, have been responsible for current taxonomic arrangement.

Monophyly of Saltator.—Hellack (1976) and Hellack and Schnell (1977) concluded that *Saltator* is not a monophyletic group. Our results also support this conclusion but for different reasons. Our biochemical data indicate that *Pitylus grossus,* traditionally treated as a close relative of *Saltator,* is more closely related to *S. coerulescens* than the latter is to three other species of *Saltator* (Fig. 1). Hellack and Schnell (1977) also found that *P. grossus* was more closely related to some saltators, including *S. coerulescens,* than those saltators were to other *Saltator* species; their branching pattern, however, differed in several ways from that shown in Fig. 1. For example, they found that *Saltator maximus* clustered with *S. coerulescens,* whereas we found that *S. maximus* was the most divergent *Saltator* analyzed. Because we analyzed only four species within this large genus, specific taxonomic recommendations are not warranted other than the merger of *Pitylus* into *Saltator.*

Relationships of Spiza americana.—Although current classifications (Morony et al. 1975, AOU 1983) follow Paynter (1970) and Hellack and Schnell (1977) in placing the Dickcissel in the Cardinalinae, others have proposed that its relationships lie elsewhere. Our results indicate that *Spiza americana* is the outgroup to all Cardinalinae analyzed except *Caryothraustes humeralis,* which is probably not a true cardinaline (see below). This was also the case in our size-corrected morphological data. This contrasts with the results of Hellack and Schnell (1977), who found that *Spiza* was the sister taxon to *Passerina* sensu strictu. Clearly, a more extensive analysis is needed to determine the relationships of *Spiza.*

Relationships of Caryothraustes humeralis.—The Yellow-shouldered Grosbeak (*C. humeralis*) has been included with the other species in the genus *Caryothraustes* since the classification of Hellmayr (1938). Hellmayr noted, however, that *C. humeralis* "probably deserves generic separation" and gave plumage and bill shape features that distinguished it from other *Caryothraustes.* Hellmayr proposed that it was "intermediate" between *Saltator,* the genus in which it was placed by Chapman (1926), and the other *Caryothraustes.* Hellack and Schnell (1977), however, retained *humeralis* in *Caryothraustes* on the basis of plumage characters, and their analysis suggested that *Caryothraustes* was most closely related to *Cyanocompsa.*

Virtually nothing was known of the natural history of *C. humeralis* until

recent fieldwork in western Amazonia revealed that the species was more widely distributed than formerly believed and that it was a scarce member of mixed-species canopy flocks composed primarily of tanagers (Remsen and Ridgely 1980, Schulenberg and Remsen 1982, Cardiff 1983, Schulenberg et al. 1984). We propose that *humeralis* might not be related to other *Caryothraustes* species because, in addition to the characters pointed out by Hellmayr (1938), *humeralis* differs from other *Caryothraustes* members in its feeding social system (quiet, inconspicuous, and solitary or in pairs in mixed-species flocks versus noisy, single-species flocks).

Our results suggest that *Caryothraustes humeralis* is not a member of the Cardinalinae. It is the outgroup to all taxa analyzed, including *Spiza,* other than the cotinga *Querula purpurata.* In terms of Nei's (1978) genetic distance, it is almost as distant from the Cardinalinae as is *Querula,* a member of a different suborder within the Passeriformes.

In the absence of tissue samples from other *Caryothraustes* species, the relationships of *humeralis* to its purported congeners cannot be ascertained at present. It is not surprising that *C. humeralis* clustered with its congener, *C. canadensis,* in the morphological phenograms uncorrected for size because overall similarities in size and shape probably contributed to the original allocation of *humeralis* to the genus *Caryothraustes.* We predict that *humeralis* will be found to be unrelated to other species in the genus *Caryothraustes.*

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LITERATURE CITED

American Ornithologists' Union. 1983. Check-list of North American birds. 6th ed. A.O.U., Washington, D.C.

Barrowclough, G. F. and N. K. Johnson. 1988. Genetic structure of North American birds. Proc. XIX Internat. Ornith. Congress, pp. 1630–1638.

Bates, J. M., T. A. Parker III, A. P. Capparella, and T. J. Davis. Observations on campo, cerrado, and forest avifaunas of eastern Dpto. Santa Cruz, Bolivia, including 21 species new to the country. Bull. Brit. Ornith. Club (in press).

Cardiff, S. W. 1983. Three bird species new for Peru, with other distributional records from northern Departamento de Loreto. Gerfaut 73:185–192.

Chapman, F. M. 1926. Distribution of bird-life in Ecuador. Bull. Amer. Mus. Nat. Hist. 55:616–1401.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

FREEMAN, S. AND W. M. JACKSON. 1990. Univariate metrics are not adequate to measure avian body size. Auk 107:69–74.

HARRIS, H. AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland Publ. Co., Amsterdam, Netherlands.

HELLACK, J. J. 1976. Phenetic variation in the avian subfamily Cardinalinae. Occ. Pap. Mus. Nat. Hist., Univ. Kansas 57:1–22.

——— AND G. D. SCHNELL. 1977. Phenetic analysis of the subfamily Cardinalinae using external and skeletal characteristics. Wilson Bull. 89:130–148.

HELLMAYR, C. E. 1938. Catalogue of birds of the Americas. Field Mus. of Nat. Hist. Publ. Zool., Series 13, Part II.

JOHNSON, N. K., R. M. ZINK, G. F. BARROWCLOUGH, AND J. A. MARTEN. 1984. Suggested techniques for modern avian systematics. Wilson Bull. 96:543–560.

MORONY, J. J., W. J. BOCK, AND J. FARRAND. 1975. Reference list of the birds of the world. Dept. Ornith., American Mus. Nat. Hist.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

PAYNTER, R. A. 1970. Subfamily Cardinalinae. Pp. 216–245 *in* Checklist of birds of the world. Vol. 13 (R. A. Paynter and R. W. Storer, eds.). Mus. Comp. Zool., Hefferman Press, Worcester, Massachusetts.

REIST, J. D. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Can. J. Zool. 64:1363–1368.

REMSEN, J. V. JR. AND R. S. RIDGELY. 1980. Additions to the avifauna of Bolivia. Condor 82:69–75.

——— AND M. A. TRAYLOR JR. 1989. An annotated list of the birds of Bolivia. Buteo Books. Vermillion, South Dakota.

RIDGELY, R. S. AND G. TUDOR. 1989. The birds of South America. Vol. 1. Univ. Texas Press, Austin, Texas.

ROBINS, J. D. AND G. D. SCHNELL. 1971. Skeletal analysis of the *Ammodramus-Ammospiza* grassland sparrow complex: a numerical taxonomic study. Auk 88:567–590.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics, VII. Univ. of Texas Publ. 7213:145–153.

ROHLF, F. J., J. KISPAUGH, AND D. KIRK. 1974. Numerical taxonomy system of multivariate statistical programs (NTSYS). State Univ. New York, Stony Brook.

SAS INSTITUTE. 1985. SAS user's guide: statistics. Statistical analysis system. SAS Inc., Raleigh, North Carolina.

SCHULENBERG, T. S. AND J. V. REMSEN, JR. 1982. Eleven bird species new to Bolivia. Bull. Brit. Ornith. Club 1982:102.

———, S. E. ALLEN, D. F. STOTZ, AND D. A. WIEDENFELD. 1984. Distributional records from the Crodillera Yanachaga, Central Peru. Gerfaut 74:57–70.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND W. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ. Texas Publ., Studies in Genetics VI, 7103:49–90.

SIBLEY, C. G. AND L. L. SHORT. 1959. Hybridization in the buntings (*Passerina*) of the Great Plains. Auk 76:443–463.

——— AND B. L. MONROE, JR. 1990. Phylogeny and classification of birds. Yale Univ. Press, New Haven, Connecticut.

SWOFFORD, D. L. 1989. PAUP: phylogenetic analysis using parsimony. User's Manual. Ill. Nat. Hist. Surv., Champaign, Illinois.

——— AND S. H. BERLOCHER. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36:293–325.

——— AND R. B. SELANDER. 1981. BIOSYS-1: a fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281–283.

WALLACE, A. 1984. Mechanisms of morphological evolution. John Wiley and Sons, New York, New York.

WEST, D. A. 1962. Hybridization in grosbeaks (*Pheucticus*) of the Great Plains. Auk 79: 399–424.

WIEDENFELD, D. A. 1978. Eco-morphology of the New World flycatchers with analysis of correlations among skeletal elements. M. S. thesis, Louisiana State Univ., Baton Rouge, Louisiana.

WILEY, E. O. 1981. Phylogenetics. John Wiley and Sons, New York, New York.

ZINK, R. M. 1983. Evolutionary and systematic significance of temporal variation in the Fox Sparrow. Syst. Zool. 32:223–238.

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